Estradiol during (analogue-)trauma: risk- or protective factor for intrusive re-experiencing?

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Abstract

Intrusions, a key symptom of posttraumatic stress disorder (PTSD), can occur as classically conditioned responses to trauma-related cues, both in the form of images and pain sensations. Women are more vulnerable to experiencing intrusions, and gonadal hormones may underlie this sex difference. Yet so far, particularly estradiol's influence on intrusions is unclear, as PTSD-symptom studies suggesting a vulnerable window for intrusions during the high estradiol-progesterone phase diverge from fear-conditioning studies suggesting a protective role of estradiol. Here, we aim to address this discrepancy and examine the effects of estradiol on intrusions while also considering stress as potential moderator.

Forty free-cycling women participated in an ecologically informed trauma-pain-conditioning (TPC) paradigm, using trauma-films and pain as unconditioned stimuli. Predictors were salivary estradiol and stress indexed by salivary cortisol and self-reported state-anxiety during TPC. Outcomes were film- and pain-intrusions occurring during daily-life in the week following TPC and a memory-triggering-task in response to conditioned stimuli 24h after TPC.

Estradiol yielded time- and stress-dependent effects on film-intrusions during daily-life: women with higher estradiol showed initially greater probability of experiencing film-intrusions, switching to lower probability toward the end of the week. This late protective effect of estradiol on film-intrusions only held for higher state-anxious women. In contrast, estradiol showed consistent protective effects on pain-intrusions during daily-life and memory-triggering-task. Together, these data suggest that high estradiol during trauma may shield women from long-term audiovisual trauma intrusions, as well as from pain-intrusions, and thereby possibly constitute a protective factor for PTSD and potentially also for chronic pain.

Keywords: classical conditioning, intrusive memories, posttraumatic stress disorder, estradiol, trauma film

1. Introduction

Intrusive memories, i.e., the distressing, involuntary, and recurrent retrieval of the traumatic event, are a core symptom of PTSD (American Psychiatric Association, 2013; Steil & Ehlers, 2000). Prevailing theories postulate that intrusions occur when individuals are exposed to cues associated with the traumatic event (Brewin et al., 2010; Ehlers et al., 2002).

Accordingly, empirical studies supported that intrusions emerge as conditioned responses (CRs) to previously neutral cues (conditioned stimuli, CSs) that have been associated with aversive stimuli (USs) (Franke et al., 2021; Streb et al., 2017; Wegerer et al., 2013). Since cue-driven re-experiencing can arise as images (e.g., image of the perpetrator), but also as perceived pain sensations (Franke et al., n.d.; Macdonald et al., 2018; Madden et al., 2016), re-experiencing may not only maintain distressing images of the traumatic event, but also perpetuate pain beyond tissue healing (Afari et al., 2014). Understanding risk or protective factors for intrusions within both domains thus seems of critical importance.

A particularly robust risk factor for PTSD (Blanco et al., 2018; Olf, 2017) and chronic pain (Mogil, 2012), but specifically also for intrusive re-experiencing (Hsu et al., 2018; Rattel, Wegerer, et al., 2019) is being a woman. Since sex differences in these disorders are particularly evident during reproductive years, female gonadal hormones are amongst the most commonly proposed candidates to explain these differences (Gogos et al., 2019; Li & Graham, 2017; Martin, 2009).

Current understanding regarding the influence of gonadal hormones on intrusions mainly comes from PTSD-symptom studies focusing on intrusion development following real trauma and analogue-trauma (i.e., a highly stressful situation induced by aversive stimuli such as trauma-films (James et al., 2016)) and from fear-conditioning studies focusing on fear-learning processes that have been implicated in the etiology and maintenance of PTSD (Garcia et al., 2018). On the one hand, PTSD-symptom studies have suggested that women

experience more intrusions when exposed to trauma during the (mid)luteal phase of the menstrual cycle, where both estradiol (E2) and progesterone (P4) levels are high (Bryant et al., 2011; Ferree et al., 2011; Ferree & Cahill, 2009). Fear-conditioning studies on the other hand generally support that women learn and retain extinction of previously CRs better under higher E2 (Garcia et al., 2018; Li & Graham, 2016, 2017; Merz et al., 2018). Importantly, successful and enduring extinction has previously been associated with attenuated post-trauma psychopathology (Norrholm et al., 2011; Rothbaum & Davis, 2003), including intrusions (Miedl et al., 2020; Rattel, Wegerer, et al., 2019). Linking fear-conditioning and PTSD-symptom studies, one study already showed that women with higher E2 levels experienced fewer daily-life intrusions, and that this effect was partially mediated by E2 facilitating extinction of CRs (Wegerer et al., 2014). Considering this apparently protective effect of E2, the fact that some studies reported greater intrusions during a menstrual cycle phase where E2 is high seems puzzling.

Several factors might explain these discrepant results. First, most PTSD-symptom studies compared women in the (mid)luteal phase with women in the follicular phase (Bryant et al., 2011; Ferree et al., 2011; Ferree & Cahill, 2009). However, investigating high E2 during the luteal phase may be problematic since P4 levels are also high and may oppose E2's effects. Specifically, while E2 enhances glutamergic and reduces GABA-ergic neurotransmission, P4 reduces glutamergic and enhances GABA-ergic neurotransmission (Barth et al., 2015). Moreover, a recent study showed that hippocampal activation was elevated when women were scanned during the pre-ovulatory phase where E2 was unopposed by P4 (i.e., high E2, low P4), but dropped during the luteal phase (Pletzer et al., 2019). Similarly, others also suggested that the otherwise inhibitory effects of unopposed E2 on the brain's arousal circuitry (Goldstein et al., 2005, 2010) disappeared during the luteal phase (Andreano & Cahill, 2010). Possibly then, results concerning the relationship between

E2 and intrusions may change when high E2 is investigated without concomitantly high P4. Noteworthy, the problem of investigating E2 together with high P4 is also present in some human fear-conditioning studies (Milad et al., 2010; Zeidan et al., 2011), but some studies investigating unopposed E2 indeed support E2-facilitated fear extinction recall (Antov & Stockhorst, 2014; Milad et al., 2007), and antagonizing effects of P4 on E2's effects (Graham & Daher, 2016).

Furthermore, PTSD-symptom studies (Ney et al., 2019; Wassell et al., 2015) tend to hypothesize that their effects stem from a high hormonal status promoting a strongly-consolidated and poorly-contextualized trauma memory trace that is readily triggered by sensory trauma-reminders (Pitman, 1989; Pitman & Delahanty, 2005). In a fear-conditioning framework, this translates to a higher hormonal status facilitating CS ("trauma-reminders") triggering US ("trauma")-representations after learning and consolidation of the original CS-US association. Yet so far, fear-conditioning studies mostly investigated the influence of gonadal hormones on fear acquisition, extinction learning and recall. While fear acquisition captures the initial learning of CS-US association, it does not tap well into the process of CSs triggering the original CS-US memory that has been consolidated into long-term memory (McGaugh, 2000). Similarly, extinction learning and recall model processes that already implicate processes such as learning of an inhibitory CS-US memory or updating of the original CS-US memory (Craske et al., 2014; Gershman & Niv, 2012). As such, whether higher unopposed E2 at the time of trauma also inhibits triggering of the consolidated original CS-US memory following fear-acquisition remains unclear.

Ample research supports E2 enhancing hippocampal-mediated memory consolidation, including contextual memories (Taxier et al., 2020). In parallel, research also supports that stress at the time of trauma strengthens memory consolidation (de Quervain et al., 2016). Following, high E2 and stress may significantly stabilize fear memories. However, effects of

a strongly consolidated fear memory on subsequent intrusions are mixed: while some showed that manipulations enhancing memory consolidation predicted intrusions after trauma (Nicholson et al., 2014; Rombold et al., 2016), others suggested the opposite (Kleim et al., 2016). Critically, one aspect that could influence this relationship is that the degree to which individuals encode and stabilize sensory elements (which later function as triggers for intrusions) and contextual details (which aid integrating the event in time and place, and thereby prevent sensory cues from automatically eliciting intrusions) varies across situations (Brewin et al., 2010; Ehlers et al., 2004; Ehlers & Clark, 2000). The ability to successfully encode contextual information is thought to be particularly decreased under extreme stress, amongst others due to loss of hippocampal function (Brewin & Holmes, 2003). In light of evidence supporting that high E2 boosts hippocampal-mediated memories (Taxier et al., 2020), and protects from stress-induced associative and declarative memory impairments (Antov & Stockhorst, 2014, 2018), the presence of high E2 may thus be especially important in preventing decontextualized, easily-triggered fear memories during highly stressful situations.

Altogether, our understanding of E2's influence on intrusion development after trauma remains unclear. Therefore, the current study investigated whether E2 in women tested during menstrual cycle phases with varying E2, but continuously low P4 (i.e., follicular phase up to three days after estimated ovulation) influenced re-activation of the consolidated trauma memory both as intrusions in daily-life and CS-elicited intrusions in the laboratory. In order to examine both film- and pain-intrusions, we extended our trauma-conditioning paradigm (Wegerer et al., 2013) by the factor pain, resulting in a fear-conditioning procedure with trauma-films (James et al., 2016) and electrocutaneous pain stimulation as USs (Trauma-Pain-Conditioning or TPC Paradigm) (Franke et al., n.d.). Main outcomes were film- and pain-intrusions occurring during (a) daily-life in the week after TPC, (b) memory-

triggering-task (MTT) in response to the CSs from TPC, presented 24h later. Predictors were salivary E2 levels, as well as stress levels indexed by salivary cortisol (CORT) levels and state anxiety during TPC. We expected that women with higher levels of E2 develop fewer film- and pain-intrusions during daily-life and MTT, and that this protective effect would hold especially for women displaying higher stress levels during analogue-trauma.

2. Methods

2.1. Participants

As part of a larger study, we tested 74 healthy women between 18 and 35 years. Exclusion criteria were smoking, medication use, cardiovascular, neuroendocrinological, and pain-related disorders, plaster allergies, reports of current psychological/psychiatric disorders, blood-injury-injection phobia, absence of self-reported physical and psychological resilience, pregnancy, current breast-feeding, as well as high (>2-3x/week) consumption of extremely violent media. We excluded participants due to drop-out ($N=3$), technical difficulties ($N=3$), and suspected brain anomaly ($N=1$). Further, given our focus on naturally-cycling women in the follicular phase up to three days after the estimated ovulation day led to the exclusion of oral-contraceptive use ($N=26$) and a woman in the mid-luteal phase, leaving a final sample of 40 women.

2.1.1. Hormonal status

Menstrual-cycle phases were estimated based on the first day of participants' last menses reported retrospectively during the first laboratory session (i.e., TPC); and the first day of participants' next menses reported upon occurrence via text-message. Accordingly, we estimated 19 participants to be in the follicular phase of the menstrual cycle (starting at menses, up to three days before estimated ovulation), and 21 participants to be in the peri-ovulatory phase (around 2 days before/after estimated ovulation). In this sample, E2 ranged

between 0.10 and 2.40 pg/ml ($M=0.91$, $SD=0.49$), and P4 ranged between 6.94 and 640.40 pg/ml ($M=77.31$, $SD=85.91$). To account for variation in P4 levels, we included P4 as a covariate in all main analyses.

2.1.2. Clinical characteristics

We assessed participants' current depression, anxiety, and stress symptomatology with the German versions of the Depression-Anxiety-Stress-Scale (Nilges & Essau, 2015) and the State-Trait-Anxiety-Inventory (Laux et al., 1981). Further, we assessed somatization symptoms during the seven days before TPC with the Screening for Somatoform Symptoms-7 (SOMS-7; Rief & Hiller, 2003). To assess PTSD-symptomatology, we used the German version of the revised Impact-of-Event Scale (Maercker & Schützwohl, 1998) applied to the individually-rated most distressing life event. As displayed in Table 1, participants' scores were within normal ranges.

Table 1. *Sample characteristics.*

	<i>M</i>	<i>SD</i>	Range in sample
Age (years)	22.50	3.34	18 - 31
DASS - D	4.15	3.97	0 - 15
DASS - A	2.55	3.17	0 - 15
DASS - S	6.30	5.87	0 - 22
STAI-T	39.45	9.14	21 - 61
SOMS-7 ICD-10 somatization index	0.63	0.86	0 - 3
IES-R	-3.17	1.58	-4.36 - 0.91

Note. Values indicating non-clinical relevance are <10 for DASS-D, <8 for DASS-A, <15 for DASS-S, < 39-40 for STAI-T (Knight et al., 1983), and <0 for IES-R. Average ICD-10 somatization scores within the general German population are $M=1.10$ ($SD=1.70$) (Rief et al., 2001). Abbreviations: DASS-D/A/S=Depression-Anxiety-Stress-Scales; STAI-T=State-Trait-Anxiety Inventory; SOMS-7=Screening for Somatoform Symptoms (7-item version); IES-R=Impact-of-Event Scale (Revised version).

2.2. Ethics statement

The study was approved by the local Ethics Committee. All participants provided written informed consent prior to participation and were reimbursed with course credit or 80 Euro.

2.3. Screening procedures

Participants completed online-questionnaires screening for inclusion criteria up to one week before the first laboratory session. Participants were asked to refrain from eating, nicotine, and caffeine for 2h prior to the study, and to avoid alcohol or excessive exercise for 24h prior to the study (Segal & Cahill, 2009).¹

2.4. Trauma-Pain-Conditioning (TPC)

Sessions started between 15:30-19:30 with participants signing informed consent, completing the pre-TPC STAI-S (Laux et al., 1981), and providing the first saliva sample.

The TPC consisted of a conditioning procedure containing trauma-films and electrocutaneous pain-stimulation as USs. Together with control conditions (neutral film-clips, no-pain stimulation), this resulted in four US conditions: aversive film-clip+pain (US_{AP}), aversive film-clip+no-pain (US_{AnP}), neutral film-clip+pain (US_{NP}), and neutral film-clip+no-pain (US_{NnP}). *Supplement1a* provides further details on the used film-clips. During one of the aversive and one of the neutral film-clips, participants received painful electrocutaneous stimulation, individually calibrated to be rated as painful between ‘6’ and ‘7’ on a scale ranging from 0 (*not painful at all*) and 10 (*maximum pain tolerance*), for a total duration of 12.5s applied in seven pulse trains with a duration of 988ms each, and an inter-stimulus interval of 400-1300ms. The first pulse always coincided with film-clip onset. *Supplement1b* details the calibration procedure. CSs were four images resembling contextual elements of each film-clip, lasted 4s and immediately preceded the respective USs; see Figure 1 for details. After TPC, participants were immediately released from the MRI-room, provided the

¹ As this study was part of a larger investigation including neural measures, participants underwent both the TPC (laboratory-session 1) and the MTT (laboratory-session 2) in the magnetic-resonance-imaging (MRI)-scanner. Data from this investigation are reported elsewhere (Franke et al., n.d.)

second saliva sample, and completed the post-STAI-S questionnaire.

Figure 1. Trauma-pain-conditioning (TPC) paradigm. TPC started with a habituation phase (A), where each CS was presented four times for 4s without being followed by an US. The acquisition phase (B) consisted of each CS being presented eight times for 4s, with a reinforcement rate of 50%. In reinforced trials, each CS was followed by one of the four corresponding USs: aversive film+pain, aversive film+no-pain, neutral film+pain, neutral film+no-pain, lasting 16s (i.e., 16s film-clip, coinciding with or without 12.5s intermittent pain stimulation). In unreinforced trials, no film-clips or pain stimulation followed the CSs. Stimuli were presented in a pseudorandom order (not more than two consecutive stimuli of the same type), with intertrial-intervals (ITIs) ranging between 12 and 16s. After habituation and acquisition, participants rated CSs for pain and valence. For stress and hormonal assessments, participants filled out the STAI-S and provided a saliva sample immediately before and after TPC. Abbreviations: CS=conditioned stimulus; US=unconditioned stimulus; N=neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P=painful stimulation; ITI= intertrial interval; STAI-S: State-Trait Anxiety Inventory-State.

2.4.1.1. Fear-conditioning ratings

Unconditioned responses (URs). Participants rated each of the four US conditions on pain (0=*not painful*, 10=*maximally tolerable*) and valence (0=*very pleasant*, 10=*very unpleasant*) at the end of acquisition.

Conditioned responses (CRs). Participants rated each CS on pain (0=*not painful*, 10=*maximally tolerable*) and valence (0=*very pleasant*, 10=*very unpleasant*) while seeing a screenshot of the respective CS at the end of acquisition.

2.4.2. Intrusion assessments

2.4.2.1. Daily-life intrusions

We assessed daily-life intrusions over seven consecutive days through an e-diary application “PsyDiary” for smartphones (MultiMediaTechnology, 2013). After TPC, we instructed participants to register intrusive memories, i.e., spontaneously occurring memories in form of pictures, sounds, feelings, or thoughts regarding the film-clips or pain stimulation, as well as sudden recurring thoughts, feelings, or physical sensations experienced while watching the film-clips or experiencing the painful stimulation. We informed participants of the possibility that film- and pain-intrusions could mingle, and instructed them to decide whether their memory concerned primarily a film-clip or the painful sensation from the experiment. Further, we instructed participants to register every intrusion, together with associated distress (rated on a visual analog scale from 0 (*not at all distressing*) to 100 (*extremely distressing*)) in the e-diary-app upon occurrence, i.e., in an event-based manner (Rattel et al., 2018). In order to monitor participants’ compliance, we sent text-message reminders for a questionnaire at 10pm each day, where we explicitly assessed whether participants reported all intrusions throughout the day. If participants indicated non-compliance, they were asked to retrospectively estimate the true number and distress separately for film- and pain-intrusions, and we substituted the event-based intrusion score by the retrospectively-estimated intrusion scores (2% of data)².

² The event-based assessment of intrusions has shown to provide equivalent data to the retrospective assessment of intrusions (Rattel, Grünberger, et al., 2019).

Since persistent PTSD is primarily linked to intrusions perceived as very distressing (Steil & Ehlers, 2000), we aimed to obtain a more clinically-relevant variable by weighting intrusions by their distress; i.e., operationalizing both visual and pain-intrusions as “intrusion load” (i.e., daily intrusion number $\times$ average distress; equivalent to the sum of daily intrusive distress).

2.4.2.2. Memory-Triggering-Task (MTT)-intrusions

In addition to daily-life-intrusions, we also aimed to measure intrusions in the laboratory in response to CSs from TPC. To secure full memory consolidation (McGaugh, 2000), participants underwent the MTT 24h after TPC. As we wanted to keep US-expectancy high during MTT, we started the session by repeating the pain calibration and showing the US_{AnP}. As displayed in Fig. 2, during MTT participants saw the CSs from the TPC without corresponding USs intermittently in a block design. After each CS-block, participants indicated the percentage of time (0=0% of the time, 10=100% of the time) they experienced memories of the film-clips from session 1 (i.e., “film-intrusions”), as well as painful bodily sensations (i.e., “pain-intrusions”) during each CS-block. In order to weight film-intrusions for associated distress, participants also indicated how distressing they experienced the film-memories (0=*not distressing at all*, 10=*extremely distressing*). In this way, akin to daily-life intrusions, we quantified film-intrusions as intrusion load (i.e., frequency $\times$ distress of film-intrusions).³

³ To reduce subjects’ burden in the MRI-scanner, we refrained from asking for distress ratings for pain-intrusions during MTT, because pain is inherently a distressing experience, and therefore we may assume that pain-intrusions were also associated with distress.

Figure 2. Memory-triggering task (MTT). CSs appeared three times for 4s within 52s-long blocks; the interval between CS presentations ranged between 6 and 18 seconds and consisted of a black screen. Following each block, participants reported the percentage of time they experienced memories of the film-clips from the first session and how distressing they perceived these film-memories to be, as well as the percentage of time experiencing painful bodily sensations. Abbreviations: CS=conditioned stimulus; N=neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P=painful stimulation; ITI= intertrial interval.

2.5. Hormonal assessments

We determined CORT, E2, and P4 levels from saliva samples collected via an unstimulated passive drool method in 15ml Greiner tubes. Samples were immediately frozen and stored at -20° until analysis. To remove particulate matter prior to analysis, samples were centrifuged twice for 15 and 10 min respectively at 3000rpm in an Eppendorf 3750 centrifuge. Whereas we analyzed CORT separately for pre- and post-TPC, we pooled pre- and post-TPC saliva samples prior to E2 and P4 analyses to account for fluctuation in hormone release and saliva production. E2 and P4 were assessed using the high sensitivity ELISA by Salimetrics with a sensitivity of 1pg/ml for E2, and 10 pg/ml for P4. Cortisol was assessed using ELISA by DeMediTec with a sensitivity of 0.019ng/ml. All hormone levels were quantified using two

duplicate measures for each sample to increase reliability, and samples with intra-assay coefficients of variability above 25% were repeated (undergoing an additional freeze/thaw cycle).

2.6. Stress indices

To represent not only the physiological, but also the emotional stress response, we used salivary CORT levels and self-reported STAI-S scores to index stress. Possibly due to the aversive nature of the experiment, we already registered relatively high CORT levels at pre-TPC (CORT-pre: $M=5.77$, $SD=3.73$; CORT-post: $M=3.68$, $SD=1.61$). In order to also capture this potential “anticipatory stress-response”, we averaged pre- and post-TPC stress (CORT/STAI-S) scores to indicate overall stress levels during memory encoding.

2.7. Statistical analyses

All statistical analyses were performed in R-Studio (RStudio Team, 2020) in R (RCore Team, 2019). For manipulation checks and main analyses, we fitted Bayesian multilevel regression models (BMLMs) (Gelman et al., 2013; Gelman & Hill, 2006) via the brms package using Stan in R-Studio (P. C. Bürkner, 2017; Carpenter et al., 2017). All models contained repeated measurements over participants (over *days* in daily-life intrusions; over *US/CS-conditions* in MTT data and manipulation checks). Thus, to account for the dependency between observations over participants, responses by the same person were modelled with varying intercepts. Rating data were fitted with ordinal (cumulative) BMLMs (P.-C. Bürkner & Vuorre, 2019). Daily-life intrusions were fitted with a hurdle lognormal distribution to account for the inflation of zero intrusions in the data (Li et al., 2011; Tooze et al., 2002). With this approach, we fitted data in two parts where (A) estimated the probability of not experiencing (i.e., zero) vs. experiencing (i.e., non-zero) intrusions (hurdle part, modeled as a Bernoulli distribution); and (B) estimated the intrusion load (i.e., severity of) intrusions >0

(lognormal part, modeled with a lognormal distribution). Finally, we modelled film-intrusions during MTT with a skewed normal distribution to account for the left-skewed distribution of the response variable (P. C. Bürkner, 2017). For an overview of fitted models, see *Supplement2*.

2.7.1. Manipulation checks

2.7.1.1. URs

To check whether Pain and Valence manipulations were successful, we analyzed URs by means of two ordinal BMLMs. Condition (US_{AP} , US_{AnP} , US_{NP} , US_{NnP}) was entered as a factor, and centered on US_{NnP} in BMLMs. In this way, we contrasted each pain condition (US_{AP} , U_{NP}) and aversive film-clip condition (US_{AP} , US_{AnP}) against a neutral film-clip, no-pain condition.

2.7.1.2. CRs

To test to what extent participants displayed CRs during TPC, we repeated the above-described UR-analyses with participants' Pain and Valence ratings to CSs, and added an interaction between Condition $\times$ Phase (habituation; acquisition) to check whether CRs increased from habituation to acquisition.

2.7.2. Main analyses: The influence of E2 and Stress on Intrusions

2.7.2.1. Daily-life intrusions

To examine whether E2 influenced daily-life intrusions in dependence of stress we fitted separate hurdle-lognormal BMLMs for film- and pain-intrusions. Predictors were E2 concentrations (pg/ml) and Stress (indexed by (I) CORT (ng/ml), and (II) STAI-S) levels from TPC, Day (i.e., experimental day on which intrusive memory was registered), as well as the interactions $E2 \times \text{Stress}(\text{CORT/STAI-S}) \times \text{Day}$. Further, we controlled for P4 levels and

TPC testing time in all models⁴. Predictors were centered and standardized before being entered in BMLMs: E2, P4, and Stress indices were centered to their respective means, and Day was centered on the first 24h day after TPC, i.e., on the second experimental day⁵. As we expected that the effect of Day (i.e., the decay) on intrusions could vary between participants, we added a varying slope for the effect of day (Barr et al., 2013).

2.7.2.2. MTT-intrusions

To examine whether E2 influenced MTT-intrusions, we fitted separate ordinal BMLMs for film- and pain-intrusions. Predictors were equivalent to those in daily-life-intrusion models, except for ‘Day’, which was substituted by ‘Condition’: during MTT, participants reported intrusions four times, once for each CS-conditions. Again, E2, P4, and Stress indices were centered to their means, and Condition was centered on CS_{NnP}. We excluded one outlier in MTT-intrusions (± 3 SDs from the mean) from analyses due to implausible/inconsistent rating, likely owed to a missed response.

2.7.3. BMLMs model summaries

For a summary of model parameters, we reported regression coefficients and 95% credible intervals (CIs; i.e., Bayesian confidence intervals). Based upon CIs, we can state that there is a 95% probability that the respective parameter falls within this interval, given the evidence provided by the data, priors, and model assumptions. Effects were considered significantly different from zero if the estimate’s 95% CIs did not include zero (this would indicate two-sided statistical significance on a 5% level). As priors we used the weakly- or non-informative default priors of *brms*, which have only negligible influence on the obtained results (P. C. Bürkner, 2017, 2018). We report Bayesian R^2 as our measure for effect sizes

⁴ Given that these covariates were of no-interest and yielded no significant effects, we refrained from reporting them in results tables to improve readability.

⁵ To allow full consolidation of the CS-US memory and assure an equal assessment period for all participants, we excluded the first intrusion assessment day (starting directly after the TPC-session), and started counting assessment days on the day after TPC.

(Gelman et al., 2019). All Bayesian models converged according to common algorithm-agnostic (Vehtari et al., 2020) and algorithm-specific diagnostics (Betancourt, 2017). There were no divergent transitions, $Rhat < 1.01$ and $ESS > 400$ for all relevant parameters.

3. Results

3.1. Manipulation-checks

In relation to the CS_{NnP} , after conditioning participants reported stronger pain sensations to CSs signaling the USs with pain-stimulation (CS_{AP} , CS_{NP}), as well as more unpleasantness regarding CSs signaling the aversive film-clip (CS_{AP} , CS_{AnP}). In addition, participants also regarded the CS_{NP} as more unpleasant than the CS_{NnP} , and reported stronger pain sensations to the CS_{AnP} than to the CS_{NnP} . See Fig.3B and *Supplement3* for details.

Figure 3. Trauma-Pain-Conditioning manipulation checks on Pain (left panels) and Valence (right panels) ratings for USs (upper panels A) and CSs (lower panels B) in each condition. Asterisks (*) indicate that coefficients are significantly different from zero, as the corresponding 95% CI did not contain zero. Abbreviations: CI=credible interval; US=unconditioned stimuli; CS=conditioned stimuli; N=neutral film-clip; A=aversive film-clip; nP=no-painful stimulation; P=painful stimulation; hab=habituation; acq=acquisition.

3.2. Daily-life intrusions

3.2.1. Film-intrusions

Analyses estimating the probability of *film-intrusion absence* in daily-life suggested no significant main effects of E2 (Fig. 4AI-II). When Stress was indexed by STAI-S (i.e., STAI-S-models), an interaction between E2×Day suggested that higher E2 levels were associated with a lower initial probability of film-intrusion absence (see Fig. 4CII). This interaction was further modulated by STAI-S levels: as displayed in Fig. 4DII, a significant E2×STAI-S×Day interaction suggested that higher E2 was associated with an initial lower probability of film-intrusion absence during the first assessment days which however switched to a slightly higher probability of film-intrusion absence in moderately-to-highly state-anxious women, but not in low state-anxious women, toward the end of the week. When Stress was indexed by CORT (i.e., CORT-models), the interactions between E2×CORT, E2×Day and E2×CORT×Day (Fig.4B-DII) were non-significant. P4 showed no significant effects on film-intrusion absence. Table 2AI-II_{hurdle} provides corresponding regression coefficients and 95%-CIs.

Results regarding *film-intrusion severity* in daily-life revealed no main-effects of E2 or interactions between E2, Stress, and Day; and P4 had no significant effects on film-intrusion severity either. See Table 2AI-II_{lognormal} and Supplemental Fig.S4 in *Supplement4* for details

3.2.2. Pain-intrusions

Analyses estimating the probability of *somatosensory-intrusion absence* in daily-life indicated that women with higher E2 levels had a higher probability of pain-intrusion absence than women with lower E2 levels (Fig.5AI-II). Non-significant E2 × Stress and E2×Day interactions suggested that the protective effects of E2 on pain-intrusions were not modulated by individuals' stress responses (Fig.5BI-II), and held across days (Fig.5CI-II). Finally,

E2×Stress×Day were also non-significant (Fig. 5DI-II). P4 had no significant effects on pain-intrusion absence. Table 2.BI-II_{hurdle} provides corresponding regression coefficients and 95%-CIs.

Analyses estimating daily-life *pain-intrusion severity* suggested no main-effects of E2, nor interactions between E2, Stress, and Day (Fig. S5, *Supplement5*). P4 had no significant effects on pain-intrusion severity. See Table 2.BI-II_{lognormal} for respective regression coefficients and 95%-CIs.

Figure 4. Estradiol effects on daily-life film-intrusions over days, considering modulatory effects of stress. Lines depict fitted values of regressions' hurdle part (i.e., estimating the probability of "zero" film-intrusions [film-intrusion absence]) predicting film-intrusions during daily-life by E2 (panel A), E2×Stress (panel B), E2×Day (panel C), and E2×Stress×Day (panel D); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying significant effects are marked by an asterisk, plots displaying non-significant results for informative purposes are marked with "n.s." on the right upper corner. Abbreviations: CI=credible interval; E2=estradiol; CORT=cortisol; STAI-S=STAI-S: State-Trait Anxiety Inventory-State; n.s. = non-significant.

Figure 5. Estradiol effects on daily-life pain intrusions over days, considering modulatory effects of stress. Lines depict fitted values of regressions' hurdle part (i.e., estimating the probability of “zero” pain-intrusions [pain-intrusion absence]) predicting pain-intrusions during daily-life by E2 (panel A), E2 × Stress (panel B), E2 × Day (panel C) and E2 × Stress × Day (panel D); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying significant effects are marked by an asterisk, plots displaying non-significant results for informative purposes are marked with “n.s.” on the right upper corner. Abbreviations: CI=credible interval; E2=estradiol; CORT=cortisol; STAI-S=STAI-S: State-Trait Anxiety Inventory-State; n.s. = non-significant.

3.3. MTT-intrusions

Across E2 analyses, in relation to the CS_{NnP}, participants reported more film-intrusions and pain-intrusions to the CS_{AP}, CS_{NP}, and the CS_{AnP} (Table 4ABI-II). See Fig.6 for details.

Figure 6. Memory-triggering-task (MTT) film-intrusions (i.e., the percentage of time of experienced film-clip memories $\times$ associated distress) and the percentage of time of experienced painful bodily sensations (pain-intrusions) in each CS-condition 24h after analogue-trauma. Asterisks (*) indicate that coefficients are significantly different from zero, as the corresponding 95% CI did not contain zero (note that because coefficients are comparable across all STAI-S- and CORT-models, for simplicity here we only plotted results from models with CORT). Abbreviations: CI=credible interval; CS=conditioned stimuli; N=neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P=painful stimulation.

3.3.1. Film-intrusions

Analyses revealed no significant main effects of E2 or interactions between E2 $\times$ Stress, or E2 $\times$ Stress $\times$ CS-conditions (Fig. 7). However, both CORT- and STAI-S-models suggested interactions between Stress $\times$ CS-conditions, in that, in comparison to the CS_{NnP}, those with higher CORT-levels reported more intrusions to the CS_{AnP} (Fig. S6BI, *Supplement6*), and those with higher STAI-S levels reported more intrusions to the CS_{NP} (Fig. S6BII, *Supplement6*). Across models, P4 did not significantly influence film-intrusions during MTT. See Table 4A for statistical parameters.

3.3.2. Pain-intrusions

In CORT-models, although results suggested no significant main-effect of E2 (Fig.8AI) or interactions between E2 $\times$ CORT (Fig. 8BI), significant interactions between E2 $\times$ CS_{AP} and

$E2 \times CS_{NP}$ revealed that higher E2 levels protected women from pain-intrusions to the CSs that did not signal the pain-stimulation (CS_{AnP}, CS_{NnP}), but yielded no significant influence on pain-intrusions to the CSs that signaled the pain-stimulation (CS_{AP}, CS_{NP}), see Fig. 8CI. Non-significant interactions between E2, CS-Conditions and CORT suggested that these effects were not modulated by CORT (Fig. 8DI). STAI-S models did not reveal significant main-effect of E2 (Fig. 8AII) or interactions between $E2 \times STAI-S$ (Fig. 8BII) either, and although interactions between E2 and CS-conditions followed the same pattern as in CORT-models, confidence intervals contained zero, and were thus uncertain (Fig. 8CII). Analyses however suggested that STAI-S-levels positively predicted pain-intrusions across CS-conditions (Fig. S7AB-II, *Supplement 7*). Finally, P4 yielded no significant effects on pain-intrusions during MTT either. Table 3B provides regression coefficients and 95%-CIs of the aforementioned effects.

Figure 7. Estradiol effects on memory-triggering-task (MTT) film-intrusions (i.e., the percentage of time of experienced film-clip memories $\times$ associated distress) over CS-conditions, considering modulatory effects of stress. Lines depict fitted values of the regressions predicting film-intrusions during MTT by E2 (panel A), E2 $\times$ Stress (panel B), E2 $\times$ CS-conditions (panel C), and E2 $\times$ CS-conditions $\times$ Stress (panel D); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying non-significant results for informative purposes are marked with “n.s.” on the right upper corner. Abbreviations: CI=credible interval; MTT=memory-triggering-task; CS=conditioned stimuli; E2=estradiol; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; N=neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P=painful stimulation; n.s. = non-significant.

Figure 8. Estradiol effects on memory-triggering-task (MTT) pain-intrusions over CS-conditions, considering modulatory effects of stress. Lines depict fitted values of the regressions predicting pain-intrusions during MTT by E2 (panel A), E2 $\times$ Stress (panel B), E2 $\times$ CS-conditions (panel C), and E2 $\times$ CS-conditions $\times$ Stress (panel D); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying non-significant results for informative purposes are marked with "n.s." on the right upper corner. Abbreviations: CI=credible interval; MTT=memory-triggering-task; CS=conditioned stimuli; E2=estradiol; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; N=no painful stimulation; A=aversive film-clip; nP=no painful stimulation; P=painful stimulation; n.s. = non-significant.

Table 2.*Bayesian multilevel model predicting daily-life (A) film-intrusions and (B) pain-intrusions by E2, (I) CORT, (II) STAI-S, and Day*

(A) Film-intrusions						(B) Somatoform Intrusions							
Hurdle			Lognormal			Hurdle			Lognormal				
<i>b</i>	95% CI		<i>b</i>	95% CI	<i>R</i> ²	95% CI	<i>b</i>	95% CI	<i>b</i>	95% CI	<i>R</i> ²	95% CI	
<i>I. CORT</i>						0.49	[0.33, 0.63]	0.39 [0.12, 0.51]					
E2	-0.55	[-1.54, 0.35]	-0.03	[-0.48, 0.43]			1.67	[0.22, 3.56]	0.53	[-0.68, 1.77]			
P4	0.34	[-0.44, 1.15]	0.05	[-0.39, 0.49]			-0.73	[-2.10, 0.44]	-0.58	[-1.76, 0.59]			
CORT	-0.69	[-1.54, 0.35]	-0.05	[-0.43, 0.32]			0.01	[-1.24, 1.27]	-0.38	[-1.28, 0.52]			
Day	0.85	[0.59, 1.15]	-0.23	[-0.34, -0.12]			0.52	[0.09, 1.05]	-0.15	[-0.72, 0.38]			
E2 × CORT	-0.58	[-1.60, 0.35]	0.09	[-0.32, 0.49]			0.20	[-1.14, 1.54]	-0.37	[-1.40, 0.64]			
E2 × Day	0.10	[-0.17, 0.39]	-0.04	[-0.16, 0.09]			-0.24	[-0.70, 0.21]	0.12	[-0.53, 0.71]			
Day × CORT	0.13	[-0.14, 0.42]	0.05	[-0.07, 0.16]			-0.22	[-0.61, 0.15]	-0.05	[-0.50, 0.43]			
Day × E2 × CORT	0.20	[-0.09, 0.50]	0.04	[-0.07, 0.16]			-0.05	[-0.46, 0.35]	-0.05	[-0.53, 0.46]			
<i>II. STAI-S</i>						0.48	[0.32, 0.63]	0.44 [0.16, 0.50]					
E2	-0.74	[-1.69, 0.15]	0.01	[-0.40, 0.43]			1.76	[0.26, 3.74]	0.29	[-1.42, 1.79]			
P4	0.32	[-0.50, 1.14]	-0.01	[-0.45, 0.44]			-0.67	[-2.07, 0.54]	-0.15	[-1.64, 1.51]			
STAI-S	-0.09	[-0.89, 0.70]	0.19	[-0.18, 0.55]			-0.51	[-1.92, 0.73]	0.57	[-0.85, 1.96]			
Day	0.92	[0.64, 1.25]	-0.21	[-0.34, -0.08]			0.62	[0.10, 1.27]	-0.09	[-1.36, 1.10]			
E2 × STAI-S	-0.11	[-0.94, 0.73]	-0.05	[-0.43, 0.32]			-0.11	[-1.62, 1.23]	0.19	[-1.23, 1.74]			
E2 × Day	0.28	[0.00, 0.58]	0.01	[-0.10, 0.12]			-0.09	[-0.59, 0.49]	-0.03	[-1.72, 1.38]			
Day × STAI-S	0.19	[-0.08, 0.47]	-0.01	[-0.14, 0.12]			-0.27	[-0.81, 0.20]	-0.22	[-1.41, 1.02]			
Day × E2 × STAI-S	0.29	[0.00, 0.58]	0.05	[-0.07, 0.17]			-0.15	[-0.68, 0.34]	-0.07	[-1.45, 1.59]			

Note. Coefficients are considered significantly different from zero if the corresponding 95% CI does not contain zero, and are highlighted in bold. For improved readability, we do not display intercepts in the current Table nor parameters concerning the control variable testing time. The models' hurdle part predicted the probability of "zero" intrusions [intrusion absence]; The lognormal part of the models predicted the amount of intrusions [intrusion severity]. Abbreviations: b = regression coefficient; CI=credible interval; E2=estradiol, CORT=cortisol; STAI-S=State-Trait Anxiety Inventory-State.

Table 3.

Bayesian multilevel model predicting laboratory CS-elicited (A) film-intrusions and (B) pain-intrusions by E2, (I) CORT, (II) STAI-S, and CS-conditions

	(A) Visual				(B) Somatosensory			
	<i>b</i>	95% CI	<i>R</i> ²	95% CI	<i>b</i>	95% CI	<i>R</i> ²	95% CI
<i>I – CORT</i>			0.53	[0.44, 0.59]			0.55	[0.46, 0.63]
E2	-1.90	[-8.50, 4.06]			-1.51	[-3.41, 0.21]		
P4	-0.03	[-4.37, 4.10]			-0.35	[-1.82, 1.08]		
CORT	0.58	[-6.16, 7.30]			0.82	[-1.13, 2.79]		
CS _{NP}	6.87	[0.37, 13.65]			2.77	[1.53, 4.13]		
CS _{AnP}	10.11	[3.42, 17.07]			2.28	[0.99, 3.61]		
CS _{AP}	12.11	[5.14, 19.40]			2.77	[1.52, 4.13]		
E2 × CORT	3.56	[-3.34, 11.38]			0.78	[-1.38, 3.03]		
E2 × CS _{NP}	2.17	[-4.47, 9.21]			1.55	[0.19, 3.05]		
E2 × CS _{AnP}	-3.37	[-10.89, 4.01]			0.41	[-1.00, 1.94]		
E2 × CS _{AP}	-1.63	[-8.87, 5.76]			1.43	[0.08, 2.93]		
CORT × CS _{NP}	2.44	[-6.01, 10.80]			-1.42	[-3.14, 0.22]		
CORT × CS _{AnP}	9.25	[0.62, 17.82]			-0.59	[-2.33, 1.14]		
CORT × CS _{AP}	5.63	[-3.90, 14.98]			-0.74	[-2.47, 0.95]		
E2 × CS _{NP} × CORT	-1.61	[-10.79, 7.18]			-1.80	[-3.68, -0.01]		
E2 × CS _{AnP} × CORT	5.44	[-4.60, 15.34]			-1.05	[-3.00, 0.78]		
E2 × CS _{AP} × CORT	-2.58	[-12.54, 6.60]			-0.93	[-2.79, 0.81]		
<i>II – STAI-S</i>			0.51	[0.43, 0.57]			0.56	[0.46, 0.63]
E2	-0.01	[-0.55, 5.27]			-1.12	[-2.81, -0.45]		
P4	-1.33	[-5.75, 3.45]			-0.56	[-1.79, 0.69]		
STAI-S	0.59	[-4.18, 5.31]			1.48	[0.25, 2.78]		
CS _{NP}	8.13	[1.66, 14.70]			2.75	[1.53, 4.11]		
CS _{AnP}	10.26	[3.76, 17.06]			1.91	[0.57, 3.33]		
CS _{AP}	12.45	[5.64, 19.47]			2.75	[1.52, 4.11]		
E2 × STAI-S	-0.79	[-5.39, 4.02]			-0.25	[-1.55, 1.08]		
E2 × CS _{NP}	1.23	[-4.86, 7.50]			1.12	[-0.22, 2.67]		
E2 × CS _{AnP}	-2.51	[-8.97, 4.06]			-0.40	[-2.05, 1.14]		
E2 × CS _{AP}	-1.81	[-8.01, 4.36]			1.13	[-0.26, 2.60]		
STAI-S × CS _{NP}	6.66	[0.38, 13.10]			-0.05	[-1.17, 1.01]		
STAI-S × CS _{AnP}	4.27	[-1.45, 9.99]			0.17	[-1.03, 1.35]		
STAI-S × CS _{AP}	3.71	[-2.07, 9.41]			-0.19	[-1.32, 0.90]		
E2 × CS _{NP} × STAI-S	-1.42	[-7.69, 4.82]			0.32	[-1.10, 1.33]		
E2 × CS _{AnP} × STAI-S	-1.73	[-7.85, 4.59]			1.09	[-0.28, 2.48]		
E2 × CS _{AP} × STAI-S	0.76	[-5.51, 6.97]			0.38	[-0.86, 1.56]		

Note: Coefficients are considered significantly different from zero if the corresponding 95% CI does not contain zero, and are highlighted in bold. For improved readability, we do not display intercepts in the current Table. Abbreviations: *b*= regression coefficient; CI=credible interval; E2=estradiol, CORT=cortisol; STAI-S=State-Trait Anxiety Inventory-State; CS=conditioned stimuli; N=neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P=painful stimulation.

4. Discussion

This study scrutinized E2 as a risk vs. protective factor for film- and pain-intrusions following analogue-trauma while considering potential modulatory effects of stress. Results pertaining pain-intrusions suggested a protective effect of E2 on pain-intrusions during daily-life and MTT regardless of participants' stress levels. MTT-results further specified this effect by suggesting that women with higher E2 responded less to safety cues than women with lower E2 levels. Results concerning film-intrusions suggested time- and stress-dependent effects of E2 on film-intrusions during daily-life. Specifically, women with higher E2 showed an initial greater probability of experiencing film-intrusions in daily-life which interestingly switched to a slightly lower probability of experiencing film-intrusions later on (i.e., from day 3) in moderately-to-highly state-anxious women with higher E2 levels.

Our finding that higher E2 levels during analogue-trauma generally protected women from experiencing pain-intrusions falls in line with our previous observation, where higher E2 predicted fewer intrusions (Wegerer et al., 2014). These effects may be understood as higher E2 protecting from CRs in the form of (pain-)intrusions; i.e., from trauma-related cues eliciting representations of the consolidated US memory. Results from MTT further specified this effect by suggesting that women with higher E2 were mostly protected from experiencing pain-intrusions to CSs which did not signal the pain-stimulation and therefore functioned as safety cues.

Interestingly, a growing literature has highlighted a role of E2 in reducing CRs to safety cues (Day & Stevenson, 2020). In specific, one study showed that women with high vs. low E2 were not only better at discriminating between safety and danger conditions, but also at inhibiting fear in the presence of safety cues (Glover et al., 2013). In line, others found that higher E2 during fear-learning mostly reduced threat expectancy and physiological responding to safety cues (CS-) (Felmingham et al., 2021; Li & Graham, 2016). Recently,

researchers also found that higher E2 protected women from generalized heightened physiological responses to CS- after fear-reinstatement⁶ (Felmingham et al., 2021). Our results add to this literature in suggesting that women yielding higher E2 levels during menstrual-cycle phases with relatively low P4 during trauma also seem to be less vulnerable to showing pain-intrusions to safety cues after consolidating the original CS-US memory.

One potential explanation for these protective effects of E2 could be related to E2 strengthening the formation and consolidation of hippocampal-dependent memories (Taxier et al., 2020). In specific, studies suggested that E2 boosts grey matter volume and, when unopposed, also activation in the hippocampus (Pletzer et al., 2018, 2019; Protopopescu et al., 2008). Besides supporting contextualized memories by providing a spatial and temporal framework for relating experiences (Lisman et al., 2017), the hippocampus has also been involved in promoting danger from safety discrimination through pattern separation processes (Besnard & Sahay, 2016) and conditioned inhibition of threat responding (Meyer et al., 2019). Thus, higher E2 might enhance the consolidation of precise memories through its actions on hippocampal functioning. Additionally, some studies have suggested that higher E2 inhibited negative emotional responding to threatening stimuli (Goldstein et al., 2005, 2010; Miedl et al., 2018). This potential stress-inhibiting effect of E2 is particularly important when considering evidence suggesting that fear spreads to a wider array of harmless stimuli when the threat (US) is more intense (Dunsmoor, Kroes, et al., 2017). Following, if women with higher E2 are more efficient at regulating negative emotional responses and experience the US as less threatening, they may be less likely to respond to cues that only *might* signal the US (such as the no-pain-stimulation signaling CSs during MTT).

⁶ Fear-reinstatement is an experimental manipulation which aims to trigger the return of a conditioned fear response after extinction by unexpectedly re-exposing individuals to the US following extinction (Myers & Davis, 2002).

Intriguingly, as opposed to the robust protective effect on pain-intrusions, we found that higher E2 conferred greater risk for experiencing film-intrusions in the first days after analogue-trauma. Considering E2's positive effects on memory consolidation (Frick et al., 2018; Kashefi & Rashidy-Pour, 2014), our finding of initial film-intrusion-enhancing effect of E2 agrees with other research observing detrimental effects of sleep-related enhanced memory consolidation specifically on early intrusions (Porcheret et al., 2015). During memory consolidation, individuals consolidate not only contextual details, but also sensory-perceptual elements encountered during trauma (Payne et al., 2008). Importantly, these sensory-perceptual cues can trigger intrusions of the traumatic event (Brewin et al., 2010; Conway, 2001; Ehlers et al., 2002, 2004; Franke et al., 2021), particularly in the early days after the event (Marks et al., 2018). In line, interfering specifically with the consolidation of these sensory-perceptual elements of the trauma memory through tasks with high visuospatial demands appears to significantly prevent intrusions (Iyadurai et al., 2019). As such, higher E2 may have increased the probability of intrusion occurrence in our study by fostering consolidation of sensory-perceptual cues associated with the analogue-trauma.

Critically, the initial greater risk of intrusions in women with higher E2 flipped to a reduced risk of intrusions in moderately-to-highly anxious women toward the end of the week. This effect again resonates with sleep research finding a protective effect of sleep on intrusion development only after the passage of three days (Kleim et al., 2016). Conceivably, by strengthening the consolidation of hippocampal-dependent memories, higher E2 may, similarly to sleep (Rothschild, 2019), secure greater availability of trauma-related contextual details. This contextual information allows individuals to properly integrate the traumatic event in place, time, and autobiographical memory base, and thereby prevents a fragmented, poorly-contextualized memory as commonly observed in PTSD patients (Brewin et al., 2010; Ehlers & Clark, 2000; Marks et al., 2018). Importantly, gist-like memory representations

increase over time, as memory precision fades (Wiltgen & Silva, 2007). Moreover, fear generalization also tends to arise with this loss of memory precision (Dunsmoor, Kroes, et al., 2017). Hence, yielding more precise, contextualized memories may turn particularly relevant after some days have passed after the traumatic event.

Noteworthy, we only observed a late protective effect of higher E2 on film-intrusions in moderately-to-highly state-anxious women. Probably, women with higher state-anxiety levels (which in the current sample highly correlated with trait-anxiety levels, $p < .001$) are more vulnerable to show stress-induced impaired hippocampal functioning (Brewin & Holmes, 2003; Pitman et al., 2012). Echoing previous findings (Antov & Stockhorst, 2014, 2018), higher E2 may have thus been especially important to secure a more precise and contextualized memory consolidation in highly anxious individuals. Given evidence suggesting that overgeneralization of threat is enhanced in anxious individuals (Duits et al., 2015; Wong & Lovibond, 2018) and by stress (Dunsmoor, Otto, et al., 2017), higher anxious individuals may yield a greater risk for overgeneralizing threat memories, and therefore be significantly more protected by a well-contextualized memory than less anxious individuals.

Curiously, we did not find any significant effects of E2 on CS-elicited film-intrusions during MTT. One noteworthy difference between intrusions assessed in daily-life and MTT relates to the nature of retrieval cues. Specifically, during MTT participants were exposed to embedded visual CS which, by depicting contextual elements from the film-clips (e.g., a beach pier resembling the pier in the beach-walk scene), probably provided cues that strongly, and perhaps invariantly, lead to retrieval of the film-clips. The fact that participants were young, healthy and perceived CSs signaling aversive vs. neutral film-clips as more unpleasant even before conditioning (See Fig. 3B), renders difficulties in retrieving precise CS-film-US representations from overt CSs rather unlikely. In daily-life however retrieval cues are weaker (e.g., a bridge resembling the pier). Consequently, film-intrusion assessment

during daily-life may have provided a significantly wider array of trauma-related cues and thereby permitted the emergence of more inter-individual differences than our MTT film-intrusion assessment. In contrast, CSs contained no features that overtly signaled the pain-US and may thus have been more ambiguous, which could explain why we observed E2 effects on pain-intrusions but not on film-intrusions. To overcome the limitation of having too overt CSs, future studies using embedded CSs may opt for using cues of different modality (e.g., odors) than the USs.

We also found differential effects of E2 on daily-life pain- and film-intrusions. Before, we argued that higher E2 may have initially enhanced intrusions by strengthening the consolidation of sensory-perceptual elements. Subsequently, cues sensory-perceptually resembling those encoded during analogue-trauma can function as triggers for intrusions (Ehlers et al., 2002; Franke et al., 2021). Although we expect that such cues also trigger pain-intrusions (Franke et al., n.d.), since humans are inclined to predominantly focus on visual information (Spence, 2009), most of the re-encountered trauma-related cues might have rather resembled elements concerning the film-US than the pain-US. Due to greater sensory-perceptual resemblance (Ehlers & Clark, 2000), participants probably more readily recalled the films and reported most cue-elicited intrusions as film-related. Therefore, the initial detrimental effects of E2 may not have applied to pain-intrusions because most sensory-perceptual cues encountered during daily-life prompted film- over pain-intrusions. Moreover, although we observed that participants reported some sensory reminder cues (e.g., muscular tension), these cues still yielded relatively little resemblance with our very specific pain-US. This may have prevented automatic triggering of pain-intrusions, unless if individuals only retained a very imprecise, poorly contextualized representation of the pain-stimulation and were more prone to over-associate harmless cues to pain, as might be the case in women with lower E2 levels. Hence, women yielding lower E2 levels during trauma could be more

vulnerable to deficits in inhibiting fear and, as we suggested here, also film- and pain-intrusions to harmless cues. Since such deficits are a core characteristic of PTSD (Jenewein et al., 2016; Jovanovic et al., 2012), it may be interesting to prospectively investigate whether lower E2 during trauma is indeed associated with a greater risk for PTSD and chronic pain symptoms, and whether this risk is conveyed by overgeneralization tendencies.

The current study yields some limitations. First, since we did not use ovulation tests to determine the exact day of ovulation, and our menstrual cycle estimation's depended on participants correctly recalling the day of their last menses/notifying us on the first day of their next menses, we cannot exclude the possibility that in some women P4 already could have opposed E2 to some degree. Further, E2 levels were in general relatively low, which could be associated with the fact that our testing sessions were during afternoons/evenings, where E2 concentrations are lower (Bao et al., 2015). To secure higher E2 levels, future studies may test in the morning, and recruit women during the pre-ovulatory phase (confirmed by ovulation tests). In order to validate our results, it would be of interest to compare this high-E2 pre-ovulatory phase to the low-E2 early-follicular phase. Further research may also investigate P4's effects on E2 by comparing women in the P4-unopposed pre-ovulatory phase with women in the P4-opposed mid-luteal phase. Second, given that participants arrived relatively stressed/anxious at the laboratory, we averaged pre- and post-analogue-trauma stress levels. Thereby, while we may have captured participants' overall stress levels during analogue-trauma, we did not consider general response tendencies (STAI-S) or potential inter-individual differences in basal CORT-levels. Future studies may overcome this problem by measuring baselines stress levels during a non-threat-inducing situation. Finally, our sample size was relatively low and may have reduced statistical power. Especially higher order interaction effects are thus to be interpreted with caution until replicated in a larger sample.

4.1. Conclusions

Results suggested that women with higher E2 during analogue-trauma were at greater risk for initial daily-life film-intrusions, but toward the end of the week anxious women with higher E2 were protected from film-intrusions. Additionally, women with higher E2 were generally protected from pain-intrusions during daily-life and in response to safety cues during MTT. This could point to a picture where higher E2 during trauma, possibly by enhancing consolidation of sensory-perceptual trauma cues, may increase women's initial risk for intrusions. However, higher E2 might concomitantly strengthen the consolidation of more precise and contextualized memories and thereby prevent intrusions in response to cues that only remotely or not at all resemble danger cues, which tends to arise especially in more anxious individuals and over time as memory precision fades. Overarchingly, we conclude that higher E2 during trauma may protect women from maintaining long-term involuntary distressing images of the traumatic event, as well as from re-experiencing pain-sensations which could pave the way for PTSD and chronic pain.

5. Acknowledgements

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Supplements

Supplement 1. Additional information on film- and pain-US

S1a. Film-clips

Film-clips were extracted from commercial movies: two film-clips yielded aversive valence, depicting severe interpersonal violence scenes (sexual assault in a tunnel; physical assault with a fire-extinguisher) from the movie “Irreversible” (Noé, 2002); another two film-clips yielded neutral valence, depicting ordinary human social interactions with no aversive content (basketball game, beach walk) from the movies “Coach Carter” (Carter, 2005) and “Mr. Jones” (Figgis, 1993). These film-clips were previously validated for use in trauma-film studies and were matched in content, valence, and arousal (Arnaudova & Hageraars, 2017).

S1b. Painful stimulation

Electrocutaneous stimulation was applied with a 7mm diameter platinum pin WASP electrode (Brainbox Ltd, Specialty Developments, Cardiff, Wales) attached to participants' inner side of the left calf and controlled by a Digitimer DS7A constant current stimulator (Digitimer Ltd, Hertfordshire, England).

Stimulation intensity was determined individually using an established stepwise calibration procedure (Rance et al., 2014). Stimulation started at 0.2mA and was incremented stepwise by 0.2mA until participants verbally reported their perceptual threshold (*sensation just noticeable*). Stimulation intensity was then further incremented by 0.2mA per trial until participants reached their pain threshold (*sensation is painful*). Subsequently, stimulation intensity was increased stepwise by 5% of the individually-determined pain threshold until participants reported their pain tolerance (*maximum pain tolerance*), where maximum pain tolerance was defined as “the moment shortly before one would want to tear the electrode off the calf, similarly to when one wants to drop a coffee cup that is too hot.” If participants did

not reach their pain tolerance after three of these intensity-increasing trials, the current stimulation intensity was increased by 15% until pain tolerance was reached.

During the Trauma-Pain-Conditioning, the applied electrical stimulation intensity corresponded to the intensity at 30% between the individual pain threshold and pain tolerance. On a scale ranging from 0 (*no sensation*) to 5 (*painful*) to 10 (*maximum pain tolerance*), individually calibrated stimulus-intensity had to be rated between 6-7. If the rating was too low or too high, stimulus-intensity was adjusted by 0.1 mA and tested repeatedly until the desired rating was achieved.

Supplement 2. Overview of fitted statistical modelsTable 2. *Overview of statistical models.*

Manipulation checks					
URs		A		Valence_US ~ Cond + (1 Subject), family=cumulative	
		B		Pain_US ~ Condition + (1 Subject), family=cumulative	
CRs TPC		A		Valence_CS_TPC ~ Cond*phase+ (1 Subject), family=cumulative	
		B		Pain_CS_TPC ~ Cond*phase (1 Subject), family=cumulative	
Intrusive memories					
Daily-life	E2	A	I	Film_ints ~ Day * E2 * CORT + P4 + TPC_testtime +	
				Hu ~ Day * E2 * CORT + P4 + TPC_testtime +	
				(1+ Day Subject), family=hurdle_lognormal	
				A	II
		Hu ~ Day * E2 * STAI-S + P4 + TPC_testtime +			
			(1+ Day Subject), family=hurdle_lognormal		
			B	I	Pain_ints ~ Day * E2 * CORT + P4 + TPC_testtime +
		Hu ~ Day * E2 * CORT + P4 + TPC_testtime +			
	(1+ Day Subject), family=hurdle_lognormal				
	B	II	Pain_ints ~ Day * E2 * STAI-S + P4 + TPC_testtime +		
Hu ~ Day * E2 * STAI-S + P4 + TPC_testtime +					
	(1+ Day Subject), family=hurdle_lognormal				
	MTT	E2	A	I	Film_ints ~ Cond * E2 * CORT + P4 + TPC_testtime +
(1 Subject), family=cumulative					
A				II	Film_ints ~ Cond * E2 * STAI-S + P4 + TPC_testtime +
					(1 Subject), family=cumulative
B			I	Pain_ints ~ Cond * E2 * CORT + P4 + TPC_testtime +	
				(1 Subject), family=skew_lognormal	
B			II	Pain_ints ~ Cond * E2 * STAI-S + P4 + TPC_testtime +	
				(1 Subject), family= skew_lognormal	

Note. Abbreviations: URs=unconditioned responses; CRs=conditioned responses; US=unconditioned stimulus; CS=conditioned stimulus; TPC=trauma-pain-conditioning; MTT=memory-triggering task; ints=intrusions; cond=condition (aversive+pain, aversive+no-pain, neutral+pain, neutral+no-pain); E2=estradiol; P4=progesterone; phase=habituation vs. acquisition

Supplement 3. Conditioning manipulation-checks

S3a. URs

Self-reported Pain analyses suggested that participants perceived the two pain-related USs (US_{AP}, US_{NP}) but interestingly also the non-pain, yet aversive-film related US_{AnP} as more painful than the US_{NnP} (US_{AP}: $b=5.53$, 95%-CI=[4.32, 6.81]; US_{NP}: $b=5.48$, 95%-CI=[4.27, 6.79]; US_{AnP}: $b=3.22$, 95%-CI=[2.18, 4.35]). Further, Valence analyses suggested that participants perceived two aversive-film-related USs (US_{AP}, US_{AnP}), as well as the neutral, though pain-related US_{NP} as more unpleasant than the US_{NnP} (US_{AP}: $b=6.12$, 95%-CI=[4.93, 7.43]; US_{AnP}: $b=6.25$, 95%-CI=[5.08, 7.57]; US_{NP}: $b=1.78$, 95%-CI=[0.97, 2.62]). See Fig.4A for details.

S3b. CRs

Self-reported Pain analyses indicated significant interactions between CS_{AP}, CS_{NP}, CS_{AnP} and Time (CS_{AP}: $b=4.22$, 95%-CI=[2.58, 5.97]; CS_{NP}: $b=4.44$, 95%-CI=[2.69, 6.29]; CS_{AnP}: $b=2.44$, 95%-CI=[0.79, 4.18]; Fig.4B). Post-hoc analyses on Pain ratings from the acquisition phase further revealed that, after acquisition, participants perceived the two pain-related CSs (CS_{AP}, CS_{NP}), and the non-pain, yet aversive-film related CS_{AnP} as more painful than the CS_{NnP} (CS_{AP}: $b=4.66$, 95%-CI=[3.34, 6.10]; CS_{NP}: $b=4.78$, 95%-CI=[3.39, 6.24]; CS_{AnP}: $b=3.07$, 95%-CI=[1.76, 4.48]). Furthermore, Valence analyses suggested significant interactions between CS_{AP}, CS_{NP}, CS_{AnP} and Time (CS_{AP}: $b=1.50$, 95%-CI=[0.36, 2.63]; CS_{AnP}: $b=1.23$, 95%-CI=[0.09, 2.33]; CS_{NP}: $b=1.74$, 95%-CI=[0.55, 2.91], see Fig. 4B). In specific, post-hoc analyses within the acquisition phase revealed that, after acquisition, participants rated the two aversive-film-related CSs (CS_{AP}, CS_{AnP}) and the neutral, but pain-related CS_{NP} as more unpleasant than the CS_{NnP} (CS_{AP}: $b=3.11$, 95%-CI=[2.22, 4.05]; CS_{AnP}: $b=3.17$, 95%-CI=[2.24, 4.12]; CS_{NP}: $b=2.27$, 95%-CI=[1.41, 3.16]). However, further post-hoc analyses within each CS-condition suggested that only CS_{NP} and CS_{AP} were rated as

significantly more unpleasant in acquisition vs habituation (CS_{AP} : $b=1.03$, 95%-CI=[0.19, 1.88]; CS_{AnP} : $b=0.72$, 95%-CI=[-0.07, 1.54]; CS_{NP} : $b=1.10$, 95%-CI=[3.99, 8.57]; CS_{NP} : $b=-0.78$, 95%-CI=[-1.67, 0.06])

Supplement 4. Non-significant effects of interest from regressions' lognormal part
estimating the severity of film-intrusions

Supplement 5. Non-significant effects of interest from regressions' lognormal part
estimating the severity of pain-intrusions

Figure S5. Lines depict fitted values of regressions' lognormal part (i.e., estimating the amount of intrusions [pain-intrusion severity]) predicting pain-intrusions during daily-life by E2 (panel A), E2 $\times$ Stress (panel B), and E2 $\times$ Stress $\times$ Day (panel C); Upper panels display models with Stress indexed by CORT, lower panels display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered E2 estimates. Plots displaying non-significant effects are plotted for informative purposes, and marked with "n.s." on the upper right corner. Abbreviations: CI=credible interval; E2=estradiol; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; n.s. = non-significant.

Supplement 6. Stress-effects on memory-triggering-task film-intrusions

Figure S6. Stress effects on memory-triggering-task (MTT) film-intrusions. Lines depict fitted values of the regressions predicting film-intrusions during MTT by Stress (panels A), Stress $\times$ Condition (panel B). Upper panels (I) display models with Stress indexed by CORT, lower panels (II) display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered CORT and STAI-S estimates. Plots displaying significant effects are marked by an asterisk, plots displaying non-significant results for informative purposes are marked with “n.s” on the right upper corner. Abbreviations: CS=conditioned stimuli; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; N=neutral film-clip; A=aversive film-clip; nP=no

Supplement 7. Stress-effects on memory-triggering-task pain-intrusions

Figure S7. Stress effects on memory-triggering-task (MTT) pain-intrusions. Lines depict fitted values of the regressions predicting pain-intrusions during MTT by Stress (panels A), Stress $\times$ Condition (panel B). Upper panels (I) display models with Stress indexed by CORT, lower panels (II) display models with Stress indexed by STAI-S. Shaded areas represent 95% credible intervals. For illustrative purposes and better appreciation, plots depict non-mean-centered CORT and STAI-S estimates. Plots displaying significant effects are marked by an asterisk, plots displaying non-significant results for informative purposes are marked with “n.s” on the right upper corner. Abbreviations: CS=conditioned stimuli; CORT=cortisol; STAI-S= STAI-S: State-Trait Anxiety Inventory-State; N=neutral film-clip; A=aversive film-clip; nP=no painful stimulation; P=painful stimulation;